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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The natural function(s) of the peptide antibiotic, gramicidin S (GS) in its producer, Bacillus brevis Nagano, was investigated. Particular attention was paid to the possible role of GS in the differentiation process: sporulation, spore properties and germination. The GS-producing parental strain and a GS-negative mutant of this strain were compared.

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FUNCTION OF THE PEPTIDE ANTIBIOTIC, GRAMICIDIN S, IN ITS PRODUCER, BACILLUS BREVIS NAGANO

by

JACQUELINE MARIE PIRET

Submitted to the Department of Nutrition and Food Science on March 17, 1981 in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Nutritional Biochemistry and Metabolism

ABSTRACT

The natural function(s) of the peptide antibiotic, gramicidin S (GS) in its producer, Bacillus brevis Nagano, was investigated. Particular attention was paid to the possible role of GS in the differentiation process: sporulation, spore properties and germination. The GS-producing parental strain and a GS-negative mutant of this strain were compared.

Sporulation rate and efficiency, sporulation-associated events (except for GS production) were similar in both strains. Mature parental and mutant spores were equally resistant to heat, ultraviolet irradiation and solvents. Germination initiation in both strains was rapid and both responded similarly to a spectrum of germinants tested. Thus the lack of GS synthesis impaired none of these properties.

Outgrowth, however, was affected. In the presence of exogenous GS, spore outgrowth was inhibited. Whereas mutant spores proceeded through outgrowth and entered vegetative growth quickly, parental spores remained in outgrowth several hours longer. Extraction of GS from the parental spores reduced the outgrowth delay. GS apparently acted from the outside on spores.

Outgrowth delay was dependent upon the concentration of GS present. There was a distinct time during outgrowth when mutant spores were no longer sensitive to exogenous GS. Outgrowth inhibition by GS was permanent. Alanine uptake experiments suggested that GS affects the entry of nutrients into the outgrowing spore.

Thesis Advisor: Dr. Arnold L. Demain
Title: Professor of Industrial Microbiology

VI. SUMMARY AND CONCLUSIONS

The role of the peptide antibiotic gramicidin S in its producer Bacillus brevis Nagano was studied. The parent strain and its GS-negative mutant, BI-7, were compared. The study focused on the postulated function of GS in differentiation: sporulation, spore properties and germination. Spores were produced in media supporting low, moderate and high GS synthesis. The extent of sporulation was inversely related to antibiotic production.

Growth and sporulation in parent and BI-7 were followed. The rate, extent and sequence of events of sporulation were similar in both strains in all media used.

The properties of mature spores of the parent and BI-7 were also compared. UV, solvent and heat resistances were determined for spores produced in a range of media. DPA contents were also measured. The absence of GS synthesis by the mutant did not impair the "quality" of its spores.

The response of spores to heat activation and to

germinants was measured by loss of absorbance of spore suspensions. The GS-negative mutant and its parent behaved similarly, regardless of the medium from which they were harvested.

Spore outgrowth, however, was affected by GS. Endogenous GS, associated with parental spores or exogenous GS, added to mutant spores, extended outgrowth by several hours. In the absence of GS or when GS was removed from spores, outgrowth was rapid. All or most of the GS was at or near the spore surface. Outgrowth inhibition by GS was probably permanent. At a distinct time during outgrowth, GS no longer exerted its effect. The uptake of labelled L-alanine by outgrowing spores was inhibited by GS. This inhibition was at a step earlier than the incorporation of L-alanine into protein.

Gramicidin S, made during sporulation by Bacillus brevis Nagano, remains associated with the sporulating, dormant and initiating organism but apparently without affecting these stages of development. During spore outgrowth, however, the antibiotic prolongs this process, inhibiting the onset of vegetative growth. In this GS-sensitive period, nutrient uptake is impaired by GS. The data suggest a mechanism of action for GS whereby it

interferes in membrane function, such as transport or energy metabolism, in outgrowing spores.

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